Supplementary information, Data S1

Materials and Methods

Tumor inoculation

WT and CCL5 KO mice were inoculated subcutaneously with 1×10^5 4T1 cells. Tumor sizes were determined every 2-3 days when tumors reached 2 cm in diameter. Tumor diameters were calculated as the square root of the length × width of the tumor as previously described [1]. Lung metastases of 4T1 tumor cells were measured as we previously described [2]. All data represent summaries of at least three independent experiments, and presented as mean ± SE.

Establishment of stable cell lines

The cell line 4T1-GM/M was obtained by stably transfecting 4T1 cells with either pSuper-M-CSF, which produced a 19-nucleotide gene-specific sequence to M-CSF (AGGAGGTGTCAGAACACTG), or pSuper-GM-CSF to GM-CSF (AAGGGCCAGGAGATTCCAC). The control cell line 4T1 was also transfected with pSuper-Scrambled as control.

Tumor infiltrating cells

Tumors were removed, minced and digested in a collagenase cocktail (1 mg/ml collagenase type 4, 20 μg/ml DNase, 10 U/ml hyaluronidase) for 2 h at room temperature [3]. Single cell suspensions were obtained by passing the digests through a 40 μm cell strainer.

Preparation of tumor associated macrophages (TAM)

Solid tumors were disaggregated by exposure to 0.3% collagenase (Sigma, St. Louis, MO, USA) for 40 min at 37 °C; 7.5×10^6 cells/ml in 10 ml RPMI1640 were put into Petri dishes. After 2 h of incubation, nonadherent cells were vigorously washed away, and the strongly adherent cells were collected and quantified as TAMs.

References

1 Ostrand-Rosenberg S, Grusby MJ, Clements VK. Cutting edge: STAT6-deficient mice have enhanced tumor immunity to primary and metastatic mammary carcinoma. *J Immunol* 2000; **165**:6015-6019.

2 Shi X, Cao S, Mitsuhashi M, Xiang Z, Ma X. Genome-wide analysis of molecular changes in IL-12-induced control of mammary carcinoma via IFN-gamma-independent mechanisms. *J Immunol* 2004; **172**:4111-4122.

3 Adler EP, Lemken CA, Katchen NS, Kurt RA. A dual role for tumor-derived chemokine RANTES (CCL5). *Immunol Lett* 2003; **90**:187-194.